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SHORT COMMUNICATION

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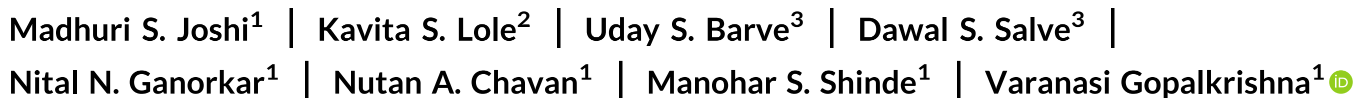
Investigation of a large waterborne acute gastroenteritis outbreak caused by group B rotavirus in Maharashtra state, India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Abstract

An acute gastroenteritis outbreak at Devli Karad village, Maharashtra, India with an attack rate of 22.6% affected mainly adolescent and adult population. The viral investigations conducted on fecal specimens of patients hospitalized indicated the presence of rotavirus B (RVB) using RNA polyacrylamide gel electrophoresis and reverse transcription polymerase chain reaction. The samples collected from the source of drinking water also showed the presence of the only RVB. Absence of other viral agents and identification of RVB of genotype G2 as the etiological agent of the acute gastroenteritis outbreak highlights, the necessity of monitoring RVB, the viral agent known for its large outbreak potential.

KEYWORDS

diarrhea, outbreak, RNA-PAGE, rotavirus B, RT-PCR

1 | INTRODUCTION

Diarrhea is the eighth leading cause of mortality and responsible for more than 1·6 million deaths.[1] Among different diarrheal etiological agents, namely viruses, bacteria, and parasites, viral gastroenteritis continues to be an important cause of morbidity and mortality globally despite the improvements in sanitation and hygiene. The clinical manifestations of viral gastroenteritis include diarrhea, vomiting, fever, anorexia, headache, abdominal cramps, and myalgia.[2] The enteric viruses associated with acute gastroenteritis include rotavirus A (RVA), rotavirus B (RVB), rotavirus C (RVC), Caliciviruses (*Norovirus* and *Sapovirus*), enteric adenovirus, human astroviruses, aichiviruses, toroviruses, coronaviruses, picobirnaviruses, enteroviruses, and Sali/Klassi viruses. Globally, RVA is the main cause of sporadic cases of acute gastroenteritis in children less than 5 years of age and outbreak cases in infants hospitalized, day care centers, and old age individuals.[3] The circulation of RVC, in individuals of all age groups has been documented in sporadic and large outbreak cases from different parts of the world.[4] In contrast, RVB infections in sporadic and outbreak cases are mainly restricted to Asian countries namely China, India, Bangladesh, Myanmar, and Nepal and largely during gastroenteritis outbreaks in adults.[5-13] Among non-rota viral agents, *Norovirus*, Astrovirus, and Adenovirus infections have been reported to be predominant and known to be responsible for different gastroenteritis outbreaks globally.[14-16] The transmission of these viruses could be water-borne, food-borne, person-to-person, and a variety of less clearly identifiable modes.

In November 2017, cases of acute gastroenteritis were reported in Devli Karad village, Nashik district, Maharashtra state of Western India. This study describes the epidemiological, environmental, and virological investigations conducted during the outbreak period.

2 | MATERIALS AND METHODS

2.1 | Clinical specimens

As per medical records maintained at the primary health care center of Tirhal, Nashik district, a total of 258 patients from Devali Karad village were admitted during the outbreak reported period. A case of acute diarrhea was defined as the occurrence of ≥3 loose stools in a day among

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the residents of Devli Karad village since 17th November 2017. This village is situated in a remote rural area connected only by roads and on around 300 km from the ICMR-National Institute of Virology (ICMR-NIV), Pune, the laboratory involved in the investigation of this outbreak. Maintaining the cold chain for storage and transport of clinical samples is very difficult in such a setting. Therefore, fecal specimens were collected from 10 representative patients admitted on 21st and 22nd November 2017 for investigation of enteric viral agents. These ten patients were between 13 and 40 years (median, 31 years) of age and six of them were males. Eight of the ten patients were from eight different families and not living in the same house and not sharing meals. Duizer et al[17] reported *Norovirus* reverse transcription polymerase chain reaction (RT-PCR) positive results, even in a single fecal specimen out of three or more are sufficient to assign it as the causative agent of the corresponding outbreak. The minimum number of specimens required to confirm RVB etiology during the outbreak could not be estimated due to the absence of data on the prevalence rate of RVB in adult diarrheal patients as well as in a healthy population.

2.2 | Enzyme-linked immunosorbent assay for detection of RVA

Each fecal specimen was suspended in 0.01 M phospate-buffered saline, pH 7.4 and mixed by vortex mixer so as to prepare 30% suspensions. The supernatant of these suspensions obtained after centrifugation were tested for the presence of RVA by using antigen capture enzyme-linked immunosorbent assay (ELISA; Premier Rotaclone, Meridian Bioscience, Inc) as per manufacturer's instructions.

2.3 | RNA extraction and viral RNA electrophoresis

Viral RNA was extracted from the supernatant of 30% fecal suspensions using TRIzol LS reagent (Invitrogen, Waltham, MA) according to the manufacturer's instructions. RNA polyacrylamide gel electrophoresis (RNA-PAGE) was used for detection of rotavirus double-stranded RNA genome segments. The electropherotyping of viral RNA was carried out in 10% PAGE at 100 V using Tris-glycine buffer and gel was stained with silver nitrate as described earlier.[18]

2.4 | RT-PCR/PCR and nucleotide sequencing

RT-PCR reaction for different viral agents was carried out by the methods as described earlier.[4,5,19-24] The specimens positive for RVB using NSP2 gene-based RT-PCR were further subjected to genotyping analysis and nucleotide sequencing using previously reported VP7 gene-specific primers.[25] Nucleotide sequence identity was determined through BLAST (www.ncbi.nlm.nih.gov/blast) analysis. The phylogenetic tree was generated with the maximum likelihood method using MEGA 6 software.[26] Nucleotide sequences of the strains examined in the study have been deposited in GenBank under the accession numbers MK515128 to MK515139.

2.5 | Collection of water samples and virus concentration

Two water samples of 5 L each was collected from two wells on 18th November 2017, the only source of drinking water in the affected area before the emergence of the outbreak. Two water samples were also collected (5 L each) from both the sources after routine chlorination treatment on 28th December 2017. All water samples were concentrated by using one-step skimmed milk flocculation method as described previously[27] and subjected to RT-PCR for detection of different viral agents.[4,5,19-24]

3 | RESULTS

Acute gastroenteritis outbreak in Devli Karad village of Western India started on 17th November 2017 and lasted for 6 days with the highest number of cases (n = 118) on 19th November (Figure 1A). Devli Karad is a village of a tribal community located in a scheduled area (these are the areas with predominance of tribal communities, as identified by the fifth schedule of the constitution of India) of Kalwan taluka, Nasik district in Maharashtra state (Figure S1) with geographical coordinates ie latitude and longitude of 20.01 and 73.78, respectively. The total population of Devli Karad was recorded to be 1140. Out of 1140 individuals, 258 of them suffered due to gastroenteritis outbreak. The age wise distribution of acute diarrheal disease cases is shown in Figure 1B. Among 258 patients, 135 were males and 96% patients were of more than 14 years of age. The case management during the outbreak period included primary health care level intervention through oral rehydration therapy at the primary health care facility. Depending on the level of dehydration, patients were treated with ORS, IV fluids, antiemetic, metronidazole, and antibiotics. None of the patients were observed to be critical.

A total of 10 fecal specimens collected from the patients (n = 10) were tested for RVA using ELISA and showed negative results. These specimens were also tested for RVC, *Norovirus* (genogroups I and II), enteric adenovirus, astrovirus, and enterovirus using RT-PCR/PCR and showed negative results.

All fecal specimens were subjected to RNA-PAGE analysis and showed RNA migration pattern of RVB (4-2-1-1-1-1-1) in all specimens. Analysis of these specimens using NSP2 gene-specific RT-PCR indicated presence of RVB specific RNA. Further VP7 genotyping and nucleotide sequencing analysis showed G2 genotype among all outbreak strains (Figure 2). Phylogenetic analysis revealed that the study strains showed 99.5% to 99.7% nucleotide identity to Indian strains and 97% to 99.7% with Indian Bangladeshi lineage strains, while Chinese lineage strains showed 90.8% nucleotide identity with study strains. Unlike strains of Indian Bangladeshi lineage with lysine at amino acid position VP7-76, all study strains showed the substitution of arginine similar to RVB strains of Chinese lineage.

The concentrated water samples of the two wells during (before chlorination) and after the outbreak period (after chlorination) were subjected to RT-PCR/PCR of RVA, RVB, RVC, *Norovirus* (genogroup I

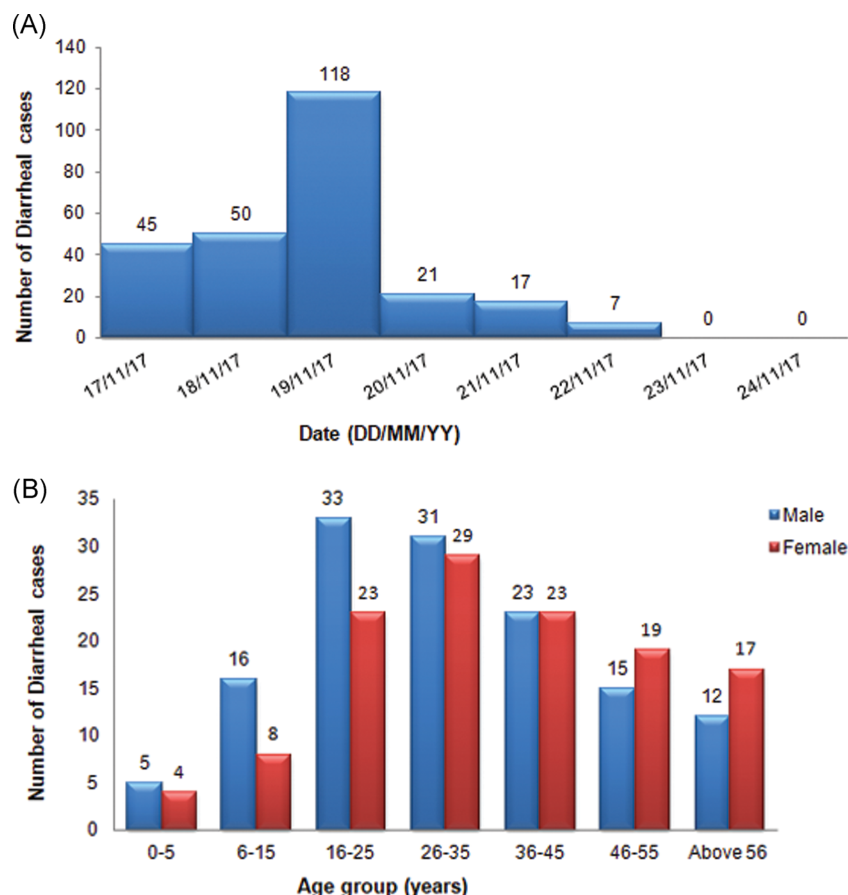

FIGURE 1 (A) Day wise and (B) age wise distribution of acute diarrheal disease cases in village Devali Karad, Maharashtra, India

and II), enteric adenovirus, astrovirus, and enterovirus. The samples were negative for all the viral agents with the exception of RVB for water samples collected during the outbreak period. Further, genotyping analysis of the VP7 gene followed by nucleotide sequencing confirmed the presence of RVB. Comparison of the RVB strains detected in water samples and fecal samples of the patients showed 100% nucleotide identity with each other. Testing of concentrated water samples collected after chlorination treatment showed negative results for all the viral agents including RVB.

4 | DISCUSSION

An acute gastroenteritis outbreak at Devli Karad village of Maharashtra state of India showed a 22.6% attack rate and was restricted mainly in individuals of more than 14 years of age (96%; Figure 1B). RNA-PAGE and RT-PCR assays coupled with nucleotide sequencing confirmed the presence of RNA of RVB in all 10 study specimens. RT-PCR/PCR for other viral pathogens showed their absence or presence below the detection limit of the assays employed. The water samples collected from two wells during the outbreak period, the only source of drinking water in the village showed the presence of RVB of genotype G2 and the absence of other viral causative agents. Therefore, RVB was presumed as the etiological agent of acute gastroenteritis outbreak occurred at Devli Karad village of Maharashtra, Western India.

Phylogenetic analysis of the VP7 gene showed clustering of the study strains with Indian Bangladeshi lineage of G2 genotype. A unique amino acid substitution at the VP7-76 position (Lysine→Arginine) was noted similar to Chinese lineage strains (ADRV/1982, WH-1/2002). This observation is in concordance with earlier outbreak reported from Pargaon, Western India in 2015.[5]

Till date, occurrence of RVB in five different gastroenteritis outbreaks has been documented from India, namely Daman (October 2000, Union territory of India), Surat (February 2004, Gujarat state), Mumbai (March 2006 and October 2006, Maharashtra state), and Sangli (May 2009, Maharashtra state).[6,7,28] The role of RVB as an etiological agent was reported for Pargaon outbreak (November 2015, Maharashtra state)[5] and in Devli Karad outbreak of the present study (November 2017, Maharashtra state). It should be noted that a total of four outbreaks out of seven were reported between October and December and two outbreaks with confirmed RVB etiology were in the month of November indicating winter seasonal pattern. However, the absence of seasonal pattern for RVB has been reported earlier from India, Bangladesh, and China.[8-11] Therefore, further large scale surveillance studies both in sporadic and outbreak settings from different parts of the country are essential to clarify the seasonal circulation of RVB.

The restriction of RVB outbreaks mainly to adolescent and adult age group individuals was observed in the present study similar to earlier reports from India and China.[5-9] Lahon et al[11] suggested that the low infection rates observed in children may be due to either low exposure of children or fecal shedding of the virus below the detection limit of the

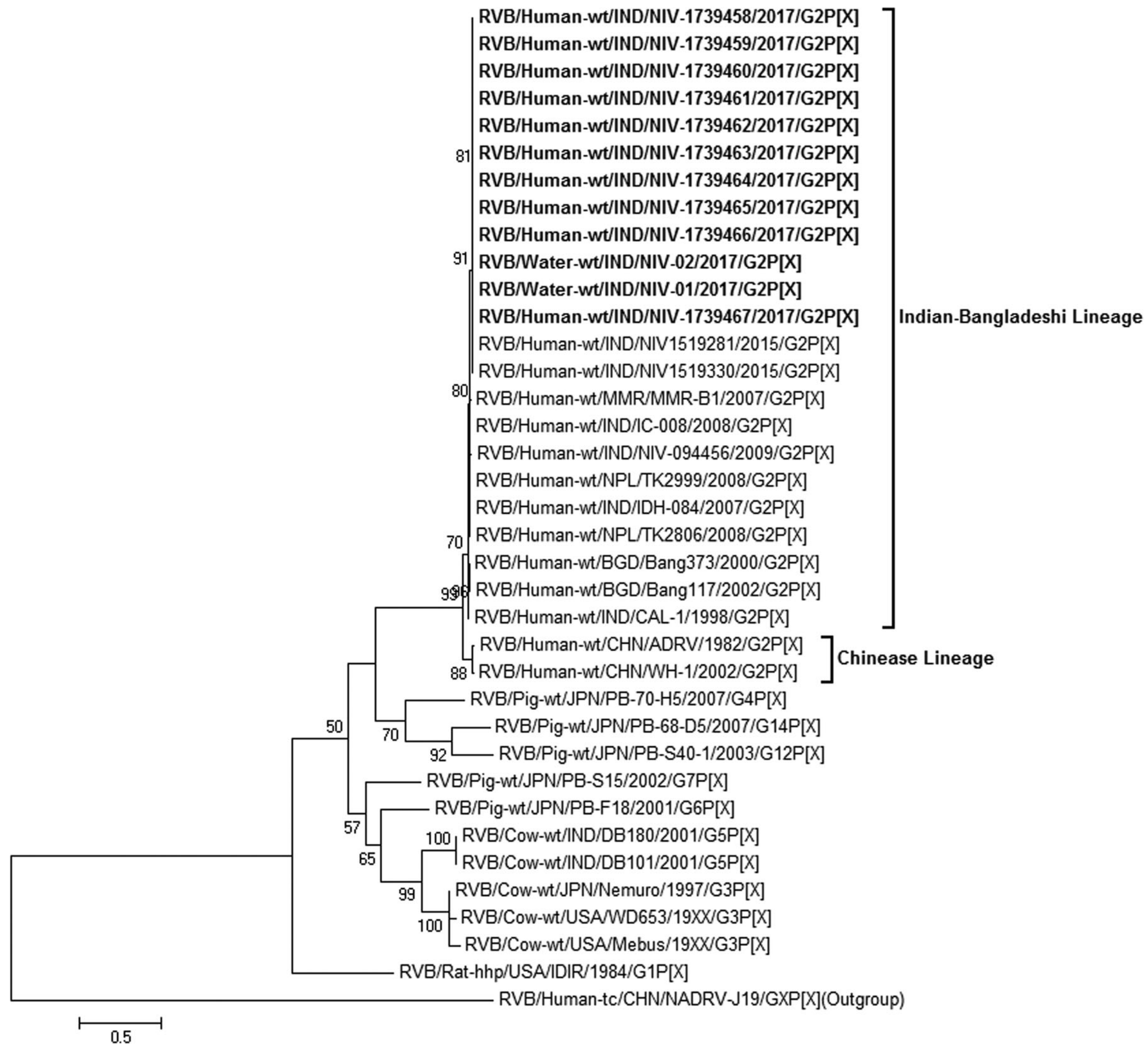

FIGURE 2 A phylogenetic dendrogram of the VP7 gene (57-782 bp) of rotavirus B (RVB) strains detected in acute gastroenteritis outbreak at Devli Karad, Maharashtra, Western India. The strains of the present study are indicated by the bold letters. The scale represents genetic distance

assay employed in the study. However, the possibility of low exposure is excluded in the present study as the source of drinking water was the same for all villagers. Similarly, RT-PCR, the only method used globally for RVB detection is known for its high sensitivity. Therefore, further studies on receptors, maternal and local immunity are necessary to understand the exact mechanism behind the resistance of the pediatric population for RVB infections. Apart from this, cross-protection due to other serotypes especially RVA, the known leading cause of gastroenteritis in children needs to be monitored in future to find out the reason behind the low attack rate of RVB in children. On the background of inclusion of RVA vaccines in national immunization schedule in India, check on the possibility of shifting or replacement of RVA with RVB or another related serogroup(s) is also necessary.

The water samples collected from the two wells were tested by the health laboratory, showed more than 16 coliforms per 100 mL of water tested indicating fecal pollution (https://www.indiawaterportal.org/articles/indian-standard-drinking-water-bis-specifications-10500-1991). From our investigation, irregular and improper chlorination was the most probable cause for the gastroenteritis outbreak, therefore an alternative arrangement of drinking water facility was provided. The regular chlorination of well water and the training for hygienic practices and water decontamination methods was given in each and every house as preventive measures. After chlorination, well water samples were tested for coliforms and showed their absence. Similarly, the absence of RVB RNA in well water samples was confirmed by concentration followed by RT-PCR.

The present study has some limitations on account of analysis restricted to smaller sample size and lack of investigations conducted on bacteria and parasites as the causative agents. However, absence of other enteric viral pathogens and presence of RVB in the fecal samples of gastroenteritis patients as well as in the source of the drinking water indicated RVB as the single viral etiological agent of the gastroenteritis outbreak at Devli Karad, Maharashtra, Western India. The present study highlights the need for routine monitoring of RVB as a potential agent of large and small outbreaks especially in India, China, Bangladesh, Myanmar, and Nepal. In addition, extensive surveillance studies both in

hospitals and communities are also necessary in different areas of the world to find out the exact disease burden due to RVB.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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